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Aug 21, 2001

DOCUMENT-IDENTIFIER: US 6277969 B1

TITLE: Anti-TNF antibodies and peptides of human tumor necrosis factor

US Patent No. (1):
6277969Detailed Description Text (54):

A suitable oligonucleotide, or set of oligonucleotides, which is capable of encoding a fragment of the variable or constant anti-TNF region (or which is complementary to such an oligonucleotide, or set of oligonucleotides) is identified (using the above-described procedure), synthesized, and hybridized by means well known in the art, against a DNA or, more preferably, a cDNA preparation derived from cells which are capable of expressing anti-TNF antibodies or variable or constant regions thereof. Single stranded oligonucleotide molecules complementary to the "most probable" variable or constant anti-TNF region peptide coding sequences can be synthesized using procedures which are well known to those of ordinary skill in the art (Belagaje, et al., J. Biol. Chem. 254:5765-5780 (1979); Maniatis, et al., In: Molecular Mechanisms in the Control of Gene Expression, Nierlich, et al., Eds., Acad. Press, NY (1976); Wu, et al., Prog. Nucl. Acid Res. Molec. Biol. 21:101-141 (1978); Khorana, Science 203:614-625 (1979)). Additionally, DNA synthesis can be achieved through the use of automated synthesizers. Techniques of nucleic acid hybridization are disclosed by Sambrook et al. (infra), and by Haynes, et al. (In: Nucleic Acid Hybridization, A Practical Approach, IRL Press, Washington, D.C. (1985)), which references are herein incorporated by reference. Hybridization wash conditions can include wash solution of 0.2.times.SSC/0.1% SDS and incubation with rotation for 10 minutes at room temperature, (low stringency wash), wash solution of prewarmed (42.degree. C.) 0.2.times.SSC/0.1% SDS and incubation with rotation for 15 minutes at 42.degree. C. (medium stringency wash) and wash solution of prewarmed (68.degree. C.) 0.1.times.SSC/0.1% SDS and incubation with rotation for 15 minutes at 68.degree. C. (high stringency wash). See Ausubel et al. (infra). Techniques such as, or similar to, those described above have successfully enabled the cloning of genes for human aldehyde dehydrogenases (Hsu, et al., Proc. Natl. Acad. Sci. USA 82:3771-3775 (1985)), fibronectin (Suzuki, et al., Bur. Mol. Biol. Organ. J. 4:2519-2524 (1985)), the human estrogen receptor gene (Walter, et al., Proc. Natl. Acad. Sci. USA 82:7889-7893 (1985)), tissue-type plasminogen activator (Pennica, et al., Nature 301:214-221 (1983)) and human term placental alkaline phosphatase complementary DNA (Keun, et al., Proc. Natl. Acad. Sci. USA 82:8715-8719 (1985)).

5, 4, 19, 22, 23

in situ

pg. 49

Column 17, line 43

line

Column 18, line 18

10-14

from an animal
line, 5-9